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Research Article

In vitro studies on antioxidant activity of black tea or *Camellia sinensis*

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Abstract: This study examined the free radical scavenging potential of crude water, ethanol and isopropanol extracts of leaves of *Camellia sinensis* (Fam: Theaceae) or black tea leaves *in vitro*. Pharmacological history of this plant inspired us to evaluate the possible antioxidant activity. Free radical scavenging activity was evaluated *in vitro* with the spectrophotometric method based on the reduction of the stable 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical. The tested extracts showed variable antioxidant activities. The highest antioxidant activity was achieved in water (48.32%) followed by ethanol (37.80%) and isopropanol (32.96%) extracts. The extracts were investigated regarding their total flavonoid content (TFC) by $A1C1_3$ reagent. The aqueous extracts of black tea leaves (TFC = 53.16 ± 1.95 µg/mg) and ethanol extracts (TFC = 46.97 ± 4.43 µg/mg) had the highest content of flavonoid whereas isopropanol extracts had minor (TFC = 10.81 ± 1.98 µg/mg) content. Furthermore, the total phenolic content of the three extracts of black tea leaves showed large variations. The water extracts of black tea leaves contained the highest total phenol content (156.00 ± 0.62 mg GAE/g extract), followed by ethanol extracts (123.33 ± 2.32 mg GAE/g extract) and isopropanol extracts (82.37 ± 1.12 mg GAE/g extract).This study suggests that leaves of black tea are the possible sources of natural radical scavengers. Thus black tea leaves could be used as natural antioxidants in the beverage, food and pharmaceutical industries that need further wide range *in vivo* studies.
Keywords: *Camellia sinensis*, antioxidant activity, DPPH assay, flavonoids

INTRODUCTION

The large generation of free radicals, particularly reactive oxygen species and their high activity plays an important role in the progression of a great number of pathological disturbances like inflammation, atherosclerosis, stroke, heart disease, diabetes mellitus, multiple sclerosis, cancer, Parkinson's disease, Alzheimer's disease, etc [1-4]. Therefore, the great interest has been recently focused on the natural foods, medicinal plants and phytocostituents due to their well-known abilities to scavenge free radicals (i.e. antioxidant power) [5,6]. Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl), and free radicals, such as the hydroxyl radical (-OH) and superoxide anion (O_2^-), are produced as normal products of cellular metabolism. Rapid production of free radicals can lead to oxidative damage to biomolecules and may cause disorders such as cancer, diabetes, inflammatory disease, asthma, cardiovascular diseases, neurodegenerative diseases, and premature aging [7]. Many medicinal plants contain large amounts of antioxidants, such as polyphenols, vitamin C, vitamin E, selenium, β-carotene, lycopene, lutein, and other carotenoids, which play important roles in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Moreover, plant secondary metabolites such as flavonoids and terpenoids play an important role in defense against free radicals [8]. Therefore, consumers should increase their intake of foods rich in antioxidant compounds that lower the risk of chronic health problems associated with the above diseases [9]. Data from various studies indicate that medicinal plants contain a wide variety of natural antioxidants, such as phenolics, flavonoids, and tannins, which possess more potent antioxidant activity than common dietary plants. Compounds responsible for such antioxidant activity can be isolated and used for prevention and treatment of free radical-related disorders [10]. Therefore, recent attention has increased to find naturally occurring antioxidants for use in food or medicine to replace synthetic antioxidants, which are being restricted due to their carcinogenicity.

Tea is produced from leaves and non-developed buds of a tea shrub having two botanical varieties: *Camellia sinensis* (Family: Theaceae) and *Camellia assamica*. Bangladesh is a tea (black tea) producer in the world followed by India (black tea), Japan (green tea) and China (different sorts of tea). Tea is the most consumed beverage in the world besides water. Based on the manufacturing process, there are three major types of tea: black (fully aerated or fermented); green (unaerated or unfermented) and oolong (partially aerated or semi fermented) [11-13]. Medicinal properties of tea were known to mankind since antiquity. Several studies have shown that tea, especially the black and green types, is very beneficial for human and animals from many

aspects. Tea has been used as a daily beverage and crude medicine throughout the world for thousands of year. Tea possesses antipyretic and diuretic effects, etc. The pharmacological effects of tea are reviewed, including antioxidative activity [14] and antimutagenic [15-17] and anticancer effects [18]. Concerning the antioxidative and anticarcinogenic effects of tea, researchers reported that green tea antioxidant (GTA) had antioxidative activity toward hydrogen peroxide and superoxide and that GTA prevented oxygen radical and hydrogen peroxide induced cytotoxicity and inhibition of intercellular communication in cell culture [19].

The aim of the present study was to evaluate and compare 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and the phenolic and flavonoid content of black tea. In addition, the study sought to determine the relationship between the DPPH radical scavenging activity and total phenolic content of different solvent extracts of this plant that might be promising sources of natural antioxidants and functional foods.

Thus, the aim of this study was to determine the antioxidant activity of black tea *in vitro*.

MATERIALS AND METHODS

Collection of black tea leaves

The aerial parts of wild-growing *Camellia sinensis* were collected at their fully mature form, from different tea gardens of Moulovibazar, Sylhet, north east part of Bangladesh at an altitude of 220 m. The parts of plant were identified by Bangladesh Council of Science and Industrial Research (BCSIR), Chittagong, Bangladesh. Samples were pure without any aromatic or additive materials. Various extracts of leaves were prepared in different solvents, then these were tested for antibacterial activity against ten pathogenic bacteria, and finally evaluated the thrombolytic potential.

Preparation of plant extracts

Fresh plant leaves were washed under running tap water and ethanol (30-40%). The leaves were cut into pieces and ground into fine powder with an electric grinder. The powder was stored in air tight bottle [20]. Aqueous extract was prepared by mixing 20.0gm of dry powder of plant leaves with 200 ml. of sterile distilled water in a round bottom flask (no.72) with occasional shaking. Before placing, the flask was washed properly and then dried. The extract was then filtered through a muslin cloth for coarse residue and finally filtered through Whatman No.1 filter paper with 150µm diameter and stored in an airtight container at 4ºC until use [21]. Ethanolic extract was prepared by mixing 50.0gm of dry powder of plant leaves with 200 ml. of 99% ethanol (Merck, Germany) and kept at room temperature for 7 days in a round bottom flask with occasional shaking. After a seven days period, the extract was filtered through a muslin cloth for coarse residue and finally filtration was done through Whatman No.1 filter paper and stored in airtight bottle at 4ºC until use [22]. In the same way the Isopropanolic extract was prepared by mixing 50.0gm of dry powder of plant leaves with 200 ml. of 98% isopropanol (Merck, Germany) and kept at room temperature for 7 days in a round bottom flask with occasional shaking. After a seven days period, the extract was filtered through a muslin cloth for coarse residue and finally filtration was done through Whatman No.1 filter paper and stored in airtight bottle at 4°C until use [23].

Chemicals

All of the chemicals used in this work were purchased from Merck (Germany), with the exception of DPPH, Flavonoid standards, gallic acid, BHA (tertbutyl-4-hydroxy-anisol), and α-tocopherol, those were purchased from Sigma-Aldrich (St. Louis, MO, USA). The chemicals were analytical grade.

Free radical scavenging activity

The free radical scavenging activity of the extracts was estimated according to the procedure described by researchers [24, 25]. Briefly, a 0.3mM solution of DPPH radical solution in ethanol 90% was prepared and then 1 ml of this solution was mixed with 2.5 ml of different concentrations of each extract (sample). The mixture was shaken and left for 30 min at room temperature in the dark, and after 30 min incubation the absorbance was then measured with a spectrophotometer at 517 nm. The antioxidant activity was calculated as the percentage of the radical scavenging activity (RSA) by the following equation:

$$RSA\% = [Acontrol – (Asample – Ablank)] / Acontrol \times 100$$

Ethanol 90% (1 ml) plus each sample solution (2.5 ml) was used as a blank. DPPH solution (1 ml) plus ethanol 90% (2.5 ml) was used as a negative control. Rutin solution (at the concentrations of 100, 50, 25, 10, 5, 2.5µg ml-1) was used as a positive control. The IC_{50} value for each sample, defined as the concentration of the test sample leading to 50% reduction of the initial DPPH concentration, was calculated from the non linear regression curve of Log concentration of the test extract (µg ml-1) against the mean percentage of the radical scavenging activity.

Total flavonoid content (TFC)

The total flavonoid content in extracts was determined according to researchers [24]. Briefly, 2.5 ml of each extract solution was mixed with 2.5 ml $AlCl_3$ reagent in ethanol 90% and allowed to stand for 40 min for reaction at room temperature. After that, the absorbance was measured spectrophotometrically at 415 nm. Ethanol 90% (2.5 ml) plus sample solution (2.5 ml) was used as a blank. Rutin was used as a reference compound. The TFC for each extarct [as µg rutin equivalents (RE) /mg of extract] was determined on the

basis of the linear calibration curve of rutin (absorbance versus rutin concentration).

Total phenolic content (TPC)

The extraction of total phenolics was performed using the Folin–Ciocalteu assay, following a standard method with some modifications [26]. In total, 100 µl of each extract (1 mg/ml) was added to a test tube containing 50 µl of the phenol reagent (1M). A further 1.85 ml of distilled deionized water was added to the solution and allowed to stand for 3 min after vortexing. Then 300 µl1 Na_2CO_3 (20% in water, v/v) was added and vortexed, and the final volume (4 ml) was obtained by adding 1.7 ml of distilled deionized water. A reagent blank was prepared using distilled deionized water. The final mixture was vortexed, then incubated for 1 h in the dark at room temperature. The absorbance was measured at 725 nm using a UV-VIS spectrophotometer. A standard curve was prepared using 0, 65.5, 125, and 250 mg/l gallic acid in methanol : water (50:50, v/v). Total phenolic values are expressed in terms of gallic acid equivalents (GAE) in milligrams per gram plant extract. All determinations were performed in triplicate.

Statistical analysis

All of the experiments were carried out in triplicate. Antioxidant activity, total phenolic content, and flavonoid content are reported as the mean ± standard deviation (SD). Significance differences for multiple comparisons were determined using oneway analysis of variance (ANOVA). Duncan's multiple range tests was used to assess the significant differences with the SPSS statistical analysis package (version 15.0; SPSS Inc., Chicago, IL, USA). Differences at $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

DPPH assay

Antioxidant tests could be based on the evaluation of lipid peroxidation or on the measurement of free radical scavenging potency (hydrogen-donating ability). The radical scavengers donate hydrogen to free radicals, leading to non toxic species and therefore to inhibition of the propagation phase of lipid oxidation. The use of DPPH radical provides an easy, rapid and convenient method to evaluate the antioxidants and radical scavengers [27- 29]. The antioxidant activity of medicinal plants is mainly related to their bioactive compounds, such as phenolics, flavonols, and flavonoids.

In this study, the antioxidant capacity of water, ethanol and isopropanol extracts of black tea leaves was systematically evaluated. The DPPH inhibition of different plant extracts is summarized in Table 1. Water extracts of leaves of black tea possessed the highest DPPH scavenging activity (48.32 ± 1.53% inhibition of the DPPH radical), followed by ethanol and isopropanol extracts (37.80 ± 1.73% and 32.96 ± 1.53%, respectively) of black tea leaves, comparable to the commercial antioxidant BHA (93.03% inhibition of the DPPH radical) and α-tocopherol (92.00%). The antioxidant activity of the leaves of *C. sinensis* could be due to the presence of wide variety of bioactive compounds, such as phenolics, flavonoids, carotenoids, and tannins in this plant.

Total flavonoid content (TFC)

Flavonoids are well-known antioxidant constituents of plants and possess a broad spectrum of chemical and biological activity, including radical scavenging properties [30]. The total content of flavonoids was evaluated and expressed as µg rutin equivalents (RE) /mg of extract. The content of flavonoids varied from 10.81 ± 1.98 to 53.16 ± 1.95 µg/mg (Table 1). The highest amount of flavonoids was found in the water extracts of leaves of black tea (53.16 ± 1.95 µg/mg), followed by ethanol extracts (46.97 ± 4.43 µg/mg) and isopropanol extracts (10.81 ± 1.98 µg/mg), indicating that these phytochemicals are likely to be responsible for the free radical scavenging activity. Flavonoids are reportedly responsible for the antioxidant activities of plants [31] through their scavenging or chelating activity [32].

Total phenolic content (TPC)

Phenolics are well established to show antioxidant activity and contribute to human health. In this study, the total phenolic content was determined using the Folin–Ciocalteu method, with gallic acid as a standard. The content of phenolics was evaluated and expressed in GAE as milligrams per gram of extract (mg GAE/g extract). The total phenolic content of the three extracts of black tea leaves showed large variations. The water extracts of black tea leaves contained the highest total phenol content (156.00 ± 0.62 mg GAE/g extract), followed by ethanol extracts (123.33 ± 2.32 mg GAE/g extract) and isopropanol extracts (82.37 ± 1.12 mg GAE/g extract). The values of total phenolic content are shown in the table 1.

Table 1: IC_{50} values of DPPH scavenging activity, total flavonoid content (TFC) and total phenolic content (TPC) of three different solvent extracts of black tea leaves

Samples	% inhibition of DPPH	TFC (µg /mg)	Total phenols (mg GAE/g extract)
Water extracts	48.32 ± 1.53	53.16 ± 1.95	156.00 ± 0.62
Ethanol extracts	37.80 ± 1.73	46.97 ± 4.43	123.33 ± 2.32
Isopropanol extracts	32.96 ± 1.53	10.81 ± 1.98	82.37 ± 1.12

CONCLUSION:

C. sinensis or black tea leaves extracts of different solvents in this research exhibited different degrees of antioxidant activity. This study indicates that black tea is one of the most effective plant in terms of antioxidant properties and can serve as natural sources to the free radical scavengers and antioxidant agents. Black tea leaves can be considered as promising sources of natural antioxidants and as possible preventative agents of some common human healthdisorders. However, the total phenolic and flavonoid content showed a weak correlation with the antioxidant activity of the investigated plant. Hence, detailed studies on the role of individual phytochemicals involved in the antioxidant activity of this plant are required for its use as functional food, beverage and in the pharmaceutical industry.

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